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OBSERVATIONS ON THE MODIFICATION IN SIZE AND SHAPE OF CHROMOSOMES DUE TO TECHNICAL PROCEDURE*

By

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With 9 Figures in the Text

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The remarkable development of cytological techniques for reliable numerical and morphological analyses of mammalian chromosomes, particularly human chromosomes, has produced by several investigators numerous papers with new and unexpected findings (CHU and GILES 1959; FORD and HAMERTON 1956; FORD, HAMERTON and JACOBS 1958; FORD, JONES, MILLER, MITWOCK, PENROSE, RIDLER and SHAPIRO 1959; FORD, JONES, POLANI, DE ALMEDIA and BRIGGS 1959; FORD, POLANI, BRIGGS and BISHOP 1959; HSU and POMERAT 1953; HUNGERFORD, DONNELLY, NOWELL and BECK 1959; LEJEUNE, TURPIN and GAUTIER 1959; LEVAN and HSU 1959; MAKINO and SASAKI 1959, 1960 a, b; SASAKI and MAKINO 1960; TIJO and LEVAN 1956; TIJO and PUCK 1958; TIJO, PUCK and ROBINSON 1959). However, certain contradictions on morphological details of chromosomes, mostly due to technical variations, are still unavoidable. For example, at present different investigators are not in complete agreement as to the correct identification of the X chromosome of man.

In the course of a study of normal somatic chromosomes of man, the author’s attention was attracted to the fact that there are considerable variations in length and arm-ratio of individual chromosomes, which might be due to technical procedures, or varying degrees of chromatid condensation. With the hope of elucidating the course of chromosome length variations, the following experiments were undertaken.

Materials and Methods

Tissues used for this investigation were taken from two to seven months old human embryos and new born rats. Splenic tissue of a golden hamster, age one month, was also employed. The tissues were cut into pieces 1—2 mm in size,

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Modification in size and shape of chromosomes

planted directly into TD-15 or TD-40 culture flasks and incubated at 37° C. McCoy's et al. (1956) synthetic medium was used with some modifications as the nutrient: it consisted of 14 different amino acids, eight kinds of vitamins in Earle's balanced salt solution and was supplemented with 20 per cent inactivated horse serum. After several days of incubation, the cells growing on the glass wall of the culture flasks were treated with a 50 × 10⁻⁸ M colchicine solution for a period varying from one to five hours at 37° C. Some cultures received no colchicine treatment. After treatment with a 0.2 per cent trypsin solution for ten minutes at 37° C the cultures were removed from the glass surface. They were then centrifuged for about five minutes at 1000 rpm. To the cell deposits dropped on glass slides were added an approximately equal volume of distilled water. After a five to ten minute interval the material was squashed with the application of acetic dahlia solution and an even pressure of the thumb. The measurements of the chromosomes were made on the basis of camera lucida drawings at a magnification of about 3000 times.

Results

1. Variations in length of chromosomes

Individual chromosomes in the metaphase stage usually exhibited a considerable variation in length due to varying degrees of condensation of composing chromatids. With the hope of clearly demonstrating this

![Variations in length of chromosomes](image)

Fig. 1. Left, Idiogram of a normal human somatic cell. Right, Four marker chromosomes (A, B, C, and D), from eight different metaphasic cells showing different degrees of chromatin variation, the author undertook some chromosome measurements. The idiogram shown in Fig. 1 represents a human somatic complement from a splenic tissue culture cell of a male embryo. The chromosomes are arranged into three morphological groups on the basis of their size and position of their centromeres. The top row includes 20 M chromosomes with median centromeres, the middle row contains 20 S chromosomes with subterminal centromeres, and the bottom row shows six T chromosomes with nearly terminal centromeres. Within each group the presumably homologous chromosomes were arranged in descending order of size. Four well distinguishable chromosomes were designated as A, B, C and D, Fig. 1. A corresponds to the largest M chromosome characterized by a nearly median centromere, B to the second largest chromosome with a submedian centromere, C to the largest T chromosome and D to the smallest S element. In order to determine the effect of colchicine on chromosome length, the slides were